Diabetic Complications Consortium

Application Title: Kidney energetics ion the development of nephropathy in type 1 diabetes

Principal Investigator: Josephine Forbes

1. Project Accomplishments:

This award was extended due to unavoidable delays in completing the compliance paperwork and with the imaging component. However, as evidenced below we have completed each component of the project and aims with the exception of the imaging.

2. Specific Aims:

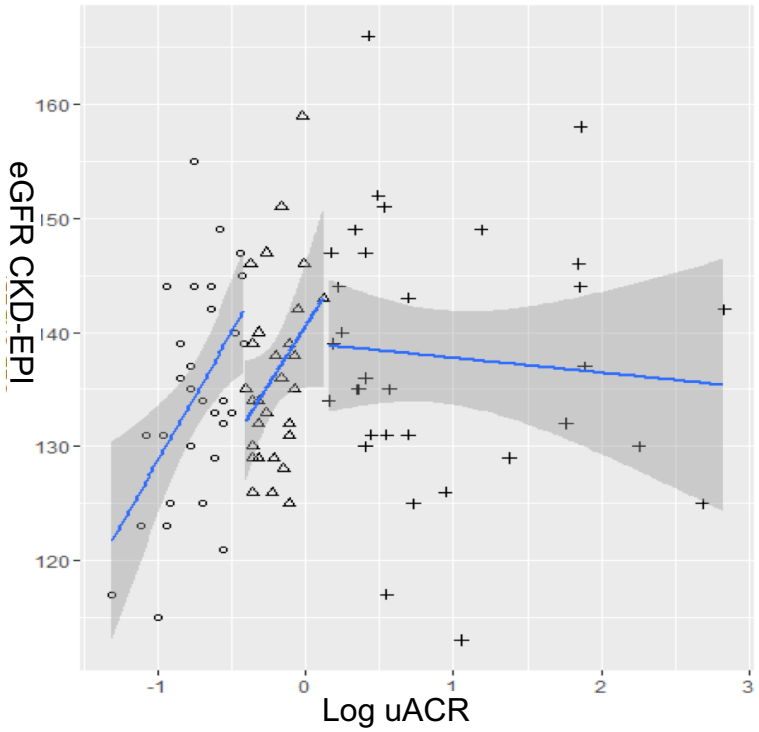
Aim 1: Obtain kidney images of fuel (lactate/fatty acids; 1H-MR) and energy storage content (ATP; 31P-MR) from young T1D individuals (15-18 yrs) with and without evidence of early kidney dysfunction and match these to urinary metabolites and urinary cell energetics.

(i) *Clinical Study*. We have completed the recruitment of and sample collection (plasma/urine/data/PBMCs) from all of the subjects in the clinical cohort. The subjects (n=100) have been divided into their baseline ACR tertiles. The unadjusted baseline data are summarised below:

	Lower Tertile (≤0.66)	Middle Tertile (0.67-1.16)	Upper Tertile (≥1.17)
N	33	33	34
Sex (n Male, %)	(21, 63.6%)	(16, 48.5%)	(17, 50%)
Age (yr)	21 (17.5-22.5)	20 (17.5-22.0)	19 (17-23.25)
Age at diagnosis (yr)	11 (7-14.5)	10 (4-12.5)	9.5 (5.5-12.25)
Diabetes duration (yr)	9.879 ± 4.904	11.42 ± 4.944	10.65 ± 5.762
HbA_1c (%)	8.0 (7.3-8.55)	8.2 (7.45-8.7)	8.45 (7.975-9.1) *
HbA_1c (mmol/mol)	63.94 (56.29-69.95)	66.13 (57.93-71.59)	68.86 (63.67-75.96) *
Fed BG (mmol/L)	11.65 (7.225-14.88)	12 (7.925-15.20)	12.55 (8.2-15.08)
Height (m)	1.75 (1.665-1.795)	1.73 (1.675-1.785)	1.71 (1.65-1.82)
Weight (kg)	80 (69-87)	74 (68.2-78.5)	74.5 (65.85-84.65)
BMI (kg/m^2)	26 (23-29.5)	24 (22.5-26.5)	24.5 (20.75-28.25)
Mean ACR (mg/mmol)	0.5 (0.415-0.57)	0.82 (0.73-0.93) *	1.73 (1.483-4.425) *†
Ur Albumin (mmol/L)	6.1 (5-10.53)	7 (5-10.9)	12.45 (5-47.38)
Ur Cr (mmol/L)	15.04 (10.56-53.50)	22.41 (10.44-56.00)	14.63 (5.095-54.75)
Plasma Cystatin C (ng/ml)	694.8 (619.7-796.6)	615.6 (585.1-754.8)	634.0 (570.0-703.5)*

Ur KIM-1 (ng/mg Cr)	48.84 (17.66-83.22)	28.40 (10.75-83.10)	31.29 (8.307-105.3)
CKD-EPI eGFR	134.7 ± 9.492	136.3 ± 8.115	137.8 ± 11.35
Schwartz eGFR	119 (104-130.5)	117 (107.5-124.5)	108 (97.5-121.8)

Table 1: Patient characteristics in the cross-sectional pilot study. Individuals with type 1 diabetes were recruited from the Mater Adolescents and Young Adult Diabetes Clinic (N=100). **P*<0.01 vs Lower Tertile; † *P*<0.05 vs Middle Tertile, ANCOVA with adjustment for age, sex and diabetes duration. HbA_{1c}, glycated haemoglobin; BMI, body mass index; eGFR, estimated glomerular filtration rate; uACR, urinary albumin:creatinine ratio. Median (IQR) or Mean ±SD.



A. Tertile: ○Lower △Middle + Upper

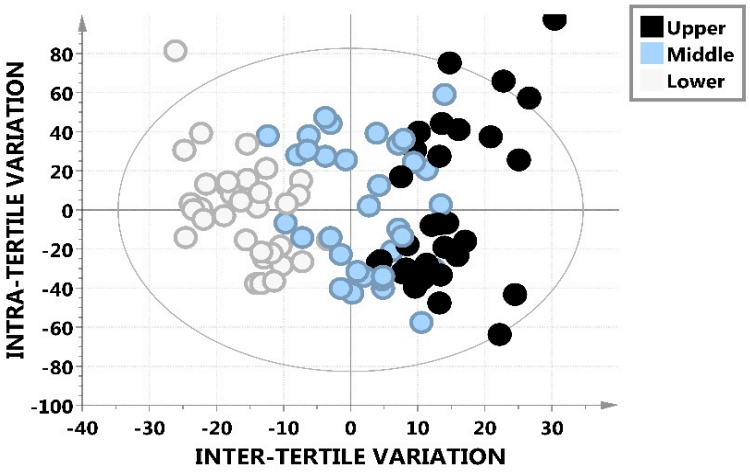
Figure 1: Young diabetic adults at risk of DKD show abnormal kidney function.
A) Relationship between log uACR and eGFR for all tertiles adjusted for age, sex and diabetes duration (N=100 with T1D).

Despite not yet showing any clinical evidence of DKD, for young diabetic adults in the higher risk tertile, lower glomerular filtration rates ($eGFR_{CKD\text{-}EPI}$) were associated with higher urinary albumin excretion (log uACR). This mirrors the relationship between these two parameters that is commonly seen in patients with chronic kidney disease (*Figure 1A*) and differentiates them from the lower risk middle and lower tertiles (*P*=0.0056; vs middle tertile). In individuals present in the upper tertile of uACR, mitochondrial function, measured as ATP linked mitochondrial respiration in circulating leukocytes, was also decreased compared with those individuals in the lower/middle uACR tertiles (*Figure 1B*).

(ii) *Urinary metabolomics*:

In a preliminary study, untargeted LC-MS-based urinary metabolomics was performed (as per Aim 1) on a cohort of diabetic individuals with varying risk for DKD (*see Table 1 and Figure 1*). Initial multivariate modelling indicated a degree of separation among tertiles of uACR based on urinary metabolite profiles.

Figure 2 (right): **Multivariate model of urinary profiling data.** Model scores plot of the analysis of urine from T1D individuals, demonstrating separation of tertile groups along the predictive component axis (x) axis, with intra-tertile variability along the orthogonal (y) axis.



Metabolites found to be differential between young adults with diabetes in the upper tertile of uACR (higher risk of DKD) and the other tertiles in this preliminary analysis include nucleotides and bases, branched chain amino acids as well as bacterial/microbiota-related intermediates (*Figure 2, above*).

(iii) *Imaging:* A new coil had been installed on the 7T Magnetom and we are just in the final stages of testing the kidney imaging. We have had almost all the patients recruited consent to MRI and so we will be able to randomly select the smaller cohort with equal numbers from the upper and lower/middle tertiles of urinary ACR.

(iv) *Metabolic flux analyses in cells*: We have obtained accurate information using flow cytometry for the urinary cell types. Unfortunately these exfoliated urinary cells had lost their capacity for oxidative phosphorylation and so we have substituted measurement of the patient PBMCs instead to assess metabolic fuel flexibility in these patients. The data (*Figure 3, below*) show that there is a change in the metabolic behaviour of the PBMCs taken from patients represented in the upper tertile of ACR, ie those most at risk of future nephropathy. These cells show a reduced capacity for energetic changes under stress.

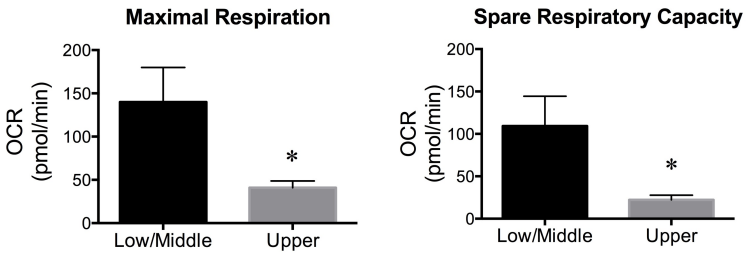


***Figure 3*:** Seahorse XF24 analyses of PBMCs during metabolic stress testing in the presence of glucose and glutamate. OCR – oxygen consumption rate.
*P<0.05 vs lower/middle tertile (n=45/20)

Aim 2: Use human kidney cells (proximal tubular cells and podocytes) derived from nephrectomy to better understand mitochondrial energy generation in type 1 diabetes.

We have completed the cellular studies including the Seahorse Flux analyses and are collating the data for publication.

3. Publications and Presentations:

1. Australian Diabetes Society 2016. Invited Keynote Speaker - Prof Josephine Forbes. Diabetic nephropathy – An energetic crisis? Gold Coast, Australia.
2. International Society of Nephrology Forefronts 2016. Diabetic nephropathy – An energetic crisis? - Prof Josephine Forbes. San Diego, USA.
3. Mater Translation of Research into Clinical Practice symposia 2016. Prof Josephine Forbes. Diabetic nephropathy – An energetic crisis? Brisbane Australia.
4. European Association for the Study of Diabetes 2017. Abstract Submitted – Mitochondrial dysfunction defines a population of young people at risk of kidney disease. * We are preparing a manuscript for submission around these data.